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*Observational Study*

**Effect of vitamin supplementation on polycystic ovary syndrome and its key pathways:** A Mendelian randomization study

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**Abstract**

**BACKGROUND**

A lot of epidemiologic investigations have explored the relationship between vitamin and polycystic ovary syndrome (PCOS). However, the effectiveness of vitamins, vitamin-like nutrients, or mineral supplementation in reducing the risk of PCOS remains a subject of debate.

**AIM**

We aimed to investigate the impact of plasma levels of vitamins A, B-12, D, E, and K on PCOS and its key pathways, namely insulin resistance, hyperlipidemia, and obesity, through Mendelian randomization (MR) analysis.

**METHODS**

Single nucleotide polymorphisms associated with vitamin levels were selected from genome-wide association studies. The primary analysis was performed using the random-effect inverse-variance-weighted approach. Complementary analyses were conducted using the weighted median, MR-egger, MR-Robust adjusted profile score, and MR-PRESSO approach.
RESULTS
The results provided suggestive evidence of a decreased risk of PCOS with genetically predicted higher levels of vitamin E (OR=0.118; 95%CI: 0.071–0.226; P<0.001) and vitamin B-12 (OR=0.753, 95%CI: 0.568–0.998, P = 0.048). An association was observed between vitamin E levels and insulin resistance (OR=0.977; 95%CI: 0.976–0.978; P<0.001). Additionally, genetically predicted higher concentrations of vitamins E, D, and A were suggested to be associated with a decreased risk of hyperlipidemia. Increased vitamin K and B-12 Levels were linked to lower obesity risk (OR=0.917, 95%CI: 0.848–0.992, P = 0.031).

CONCLUSION
The findings of this MR study suggest a causal relationship between increased vitamin A, D, E, K, and B-12 values and a reduced risk of PCOS or its primary pathways.

INTRODUCTION
Polycystic ovary syndrome (PCOS) is a diverse endocrine disease that affects a large number of sexually mature women globally [1,2]. The prevalence of PCOS according to the diagnostic criteria ranges from 6 to 10% [3]. Patients with PCOS are at an increased risk of diabetes mellitus, atherogenic dyslipidemia, systemic inflammation, hypertension, and coagulation disorders [4,5].

PCOS arises from a combination of hereditary and epigenetic vulnerability, insulin resistance, and adiposity-related mechanisms [6,7]. Modifying one’s lifestyle is among the recommended options for PCOS treatment and is highly advised for women seeking to improve their quality of life [8]. In recent years, there has been growing interest in nutritional supplements [9,10]. However, the potential of vitamin, vitamin-like nutrients, or mineral supplementation to reduce the risk of PCOS remains debatable. A meta-analysis found no evidence that vitamin D supplementation improved or alleviated metabolic and hormonal dysregulations in PCOS [11]. Nevertheless, conclusive
conclusions cannot be drawn at this point, and vitamin K may be a viable option for alleviating oxidative stress and improving glycemic control in PCOS [12].

The supplementation of specific nutrients and complementary treatments may improve the health conditions of women with PCOS by modulating critical pathways implicated in PCOS development, such as insulin signaling, insulin resistance, and lipid metabolism. However, observational studies largely constitute the primary evidence regarding the correlation between vitamin supplements and PCOS, which can be influenced by confounding or reverse causation. Mendelian randomization (MR) has emerged as an effective technique to identify the causal relationship of risk factors with diseases by using genetic variants as instrumental variables (IVs) [13]. MR enables stronger causal inferences than typical observational studies due to the random assignment of genetic variations during conception between parents and offspring.

To date, no MR analysis has explored the causal effect of vitamin supplements on PCOS. In this study, we aimed to conduct a two-sample MR analysis to assess the impact of plasma levels of vitamins A, B-12, D, E, and K on PCOS and its key pathways, namely insulin resistance, hyperlipidemia, and obesity.

MATERIALS AND METHODS

Study design

Vitamin supplementation has the potential to improve health outcomes in women with PCOS by influencing crucial pathways such as insulin resistance, lipid metabolism, and obesity. We conducted a two-sample MR analysis to identify the effect of plasma levels of vitamins A, B-12, D, E, and K on PCOS and its associated pathways, including insulin resistance, hyperlipidemia, and obesity. Figure 1 provides a detailed overview of the study design.

Study Participants

The genetic association data for vitamin D were analyzed using blood samples obtained twice from the UK Biobank, a major population cohort [14] comprising volunteers aged
37-73 from 22 evaluation centers across the UK, aiming to enhance disease prevention.

Genetic association data for vitamin B-12 were obtained from sequencing initiatives in Iceland and Denmark involving European populations, explaining 5.1% of the variation in circulating vitamin B-12 Levels. Genetic instruments for vitamin A and E concentration were obtained from three cohorts: the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study cohort; Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial Study; and the Nurses' Health Study (NHS). Data for vitamin K were obtained from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium Nutrition Working Group, which involved 2,138 individuals. Genetic correlations were examined using linear models adjusted for key components, sex, age, and study-specific factors. To mitigate potential bias stemming from ancestry, participation was limited to individuals of European heritage. All analyses of this study were based on publicly accessible databases; thus, no additional ethical approval or informed consent was required. The genome-wide association studies (GWAS) included in the analysis are presented in Table 1.

Outcome data source

The primary measure in this MR study was PCOS. Table 1 provides a summary of the specific sources of outcome data. Summary statistics for PCOS in individuals of European ancestry were obtained from the FinnGen Biobank consortium, which includes 118,870 participants. The FinnGen project is a unique research endeavor that integrates genetic data with digital healthcare information from over 500,000 Finnish biobank participants. Hyperlipidemia, obesity, and insulin resistance are key pathways of PCOS. Hyperlipidemia and obesity data were also sourced from the FinnGen Biobank consortium. Insulin resistance data were retrieved from the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC), involving up to 37,037 participants.

Genetic instrument for vitamin concentration
Single nucleotide polymorphisms (SNPs) associated with vitamins D, E, A, and B-12 were defined at the genome-wide significance threshold \( (P<5 \times 10^{-8}) \). Owing to the limited number of SNPs for vitamin K, SNPs at a level of genome-wide significance with \( P<5 \times 10^{-6} \) were chosen as IVs. To ensure instrument validity, SNPs were filtered within a 1000 kb window with an \( r^2<0.01 \) threshold \(^{[21]}\). Through a search of the GWAS Catalog (https://www.ebi.ac.uk/gwas/), we identified pleiotropic IV SNPs associated with any confounding factor related to the outcome. Estimates of the effects of these vitamin-related genetic variations on outcome datasets were collected. Additionally, SNP harmonization was conducted to restore allele orientation. The final selection of SNPs used in this MR is presented in Supplementary Tables 1–20.

**SNP-based Mendelian randomization estimates**

The primary analyses were performed using the random-effect inverse-variance-weighted (IVW) approach assuming all SNPs as valid IVs. The IVW method is considered highly reliable when there is no evidence of directional pleiotropy among the selected IVs \(^{[22]}\). Complementary analyses were conducted using the weighted median \(^{[23]}\) and MR-Egger \(^{[23]}\) methods as supplements to IVW. The weighted median model generates consistent causal findings when over 50% of the weights are derived from valid SNPs. The MR-Egger regression method can detect and adjust for directional pleiotropy \(^{[24]}\).

Furthermore, we performed MR-Robust adjusted profile score (MR-RAPS) using the “Huber” loss function to model a random-effect distribution of the pleiotropic effects of genetic variants \(^{[25]}\). Additionally, the MR-PRESSO approach was used to identify outlier SNPs and provide causal estimations after removing probable outliers, assuming that the employed SNPs are valid \(^{[26]}\).

**Heterogeneity and pleiotropy analysis**

We used Cochran’s Q test to analyze the heterogeneity of the estimations from each SNP. When there was no statistically significant heterogeneity \( (P>0.05) \), we used the
fixed-effect model; however, the random-effect model was used to produce highly conservative estimations. Pleiotropy analysis was conducted using the MR-Egger intercept test. A zero intercept for MR-Egger (P>0.05) indicates no presence of pleiotropic bias [27].

All tests were performed using the statistical program “R” v3.5, with the “TwoSampleMR,” “MR-PRESSO,” “Mr.raps,” and “Forestplot” packages. All analyses were two-sided, and statistical significance was set at P<0.05.

RESULTS

Association of vitamin E supplementation with PCOS and its key pathways

In the fixed-effect IVW estimations, genetically projected higher values of vitamin E were associated with a reduced risk of PCOS (Figure 2, Table S21). For 1-SD increase in genetically projected vitamin E concentrations, the combined OR was 0.118 (OR=0.118; 95%CI: 0.071–0.226; P<0.001). The association remained consistent in complementary analyses using random-effect IVW and weighted median techniques. Higher vitamin E levels were correlated with a decreased risk of hyperlipidemia (OR=0.259; 95%CI: 0.111–0.608; P=0.002) and insulin resistance (OR=0.977; 95%CI: 0.976–0.978; P<0.001). However, no impact of vitamin E on obesity was observed via the fixed-effect IVW approach.

Association of vitamin D supplementation with PCOS and its key pathways

Genetically anticipated higher vitamin D concentrations were suggestive of a decreased risk of hyperlipidemia, as indicated via the random-effect IVW approach (OR = 0.749, 95%CI: 0.592–0.948, P = 0.016; Figure 3, Supplementary Table 21). The results remained consistent in the fixed-effect IVW approach (OR=0.749, 95%CI: 0.647–0.868, P = 0.001). However, other complementary analyses yielded negative results. Additionally, all MR methods did not support a link between genetically projected vitamin D concentrations and PCOS, obesity, and insulin resistance.

No evidence of directional pleiotropy was observed, but heterogeneity was present for vitamin D analysis on the key pathways of PCOS (Table 2). Furthermore, outlier SNPs
were identified using the MR-PRESSO test, and the causal effect estimates of vitamin E on the risk of PCOS and its key pathways were not statistically significant (Table 3).

**Association of vitamin K supplementation with PCOS and its key pathways**

Figure 4 presents the MR estimation for the association of vitamin E supplementation with PCOS and its key pathways. According to the fixed-effect IVW method, increased vitamin E levels were associated with a reduced risk of obesity (OR=0.917, 95% CI: 0.848–0.992, \(P = 0.031\); Figure 4, Supplementary Table 21). However, no causal effect of vitamin E on PCOS, hyperlipidemia, and insulin resistance was observed. No evidence was observed of horizontal pleiotropy (P-value for intercept > 0.05; Table 2) or heterogeneity as measured using Cochran’s Q test (P-value for Cochran’s Q > 0.05; Table 3).

**Association of vitamin B-12 supplementation with PCOS and its key pathways**

The fixed-effect IVW estimations suggested a link between genetically anticipated higher vitamin B-12 levels and a lower risk of PCOS (OR=0.753, 95% CI: 0.568–0.998, \(P = 0.048\)) and obesity (OR=0.917, 95% CI: 0.843–0.995, \(P = 0.037\); Figure 5, Table S21). However, no correlation was observed between vitamin B-12 levels and hyperlipidemia or insulin resistance.

No evidence of horizontal pleiotropy (P-value for intercept > 0.05; Table 2) or heterogeneity as indicated by the Cochran’s Q test (P-value for Cochran’s Q > 0.05; Table 3) was found. After correcting for the outline SNPs, the causal effect estimates of vitamin B-12 on the risk of PCOS (\(P = 0.005\)) and obesity (\(P = 0.048\)) remained statistically significant (Table 3).

**Association of vitamin A supplementation with PCOS and its key pathways**

The IVW estimate showed a significant association between genetically predicted vitamin A levels and hyperlipidemia risk (OR=0.287, 95% CI: 0.258–0.320, P<0.001; Figure 6, Table S21). This association was consistent with complementary analyses using the random-effect IVW method. However, no statistically significant causal effect estimates of vitamin A on the risk of PCOS, obesity, and insulin resistance were observed.
DISCUSSION

Supplementation with individual nutrients may improve health outcomes in women with PCOS by altering crucial PCOS-related pathways, such as insulin signaling, insulin resistance, and lipid metabolism. This MR analysis, based on large-scale genetic consortia, provides suggestive evidence supporting a causal effect of higher vitamin E and B-12 levels on a decreased risk of PCOS. Our findings indicated that genetically predicted levels of vitamin K and B-12 were related to a lower risk of obesity. Additionally, genetically predicted higher levels of vitamin E, D, and A were suggestively associated with a decreased risk of hyperlipidemia, while higher vitamin E levels were suggestively linked to a lower risk of insulin resistance.

Previous studies have employed cross-sectional, case-control, and cohort designs to investigate the association between vitamin supplementation and the risk of PCOS. However, the findings remain disputed. For instance, Silvia Savastano found that patients with PCOS had lower levels of vitamin D compared with controls [28]. S Hahn demonstrated a link between low serum 25-hydroxyvitamin D values, insulin resistance, and obesity in women with PCOS [29]. However, a meta-analysis of 30 trials did not provide evidence that vitamin D supplementation reduces or alleviates metabolic and hormonal dysregulations in PCOS [11]. This discrepancy may be attributed to the limitations of observational investigations, which are prone to residual confounding and imprecise measurements of confounders. In contrast, MR analysis offers highly accurate causal conclusions by leveraging the random assignment of genetic variations from parents to children.

A recent systemic review of 12 articles indicated that vitamin E supplementation improves lipid profile, reduces insulin levels, and decreases HOMA-IR values [30, 31]. This is consistent with our findings that suggest increased vitamin E concentrations are associated with a decreased risk of PCOS, hyperlipidemia, and insulin resistance. The anti-oxidative property of vitamin E, along with its effects on oxidative stress metrics, may explain its positive effects on lipid profile enhancement and insulin.
resistance. Vitamin E acts as a substantial fat antioxidant, neutralizing peroxyl radicals and preventing the oxidation of polyunsaturated fatty acids. Coenzyme Q10 is often supplemented together with vitamin E due to its synergistic roles in sustaining mitochondrial activity and integrity. Foreign studies have shown favorable effects of coenzyme Q10 and vitamin E supplementation on blood insulin, HOMA-IR, and total testosterone levels in women with PCOS.

The role of vitamin B-12 on PCOS remains unclear. B-group vitamins are responsible for breaking down Hcy in the blood, which is associated with insulin resistance. However, our study provides no evidence of a causal effect of vitamin B-12 on insulin resistance. A randomized controlled trial with B-group vitamin supplementation indicated a reduction in Hcy concentrations but no changes in insulin resistance.

Lipid metabolism is a key pathway in PCOS. Our MR analysis reveals that genetically predicted higher levels of vitamins E, D, and A are suggestively associated with a decreased risk of hyperlipidemia. This finding suggests the potential benefits of supplementing these vitamins. However, further functional studies in vivo are necessary to explore the underlying mechanisms. Additionally, adipose tissue plays a role as a metabolic and endocrine organ, and its overabundance can lead to alterations in body homeostasis and vitamin deficiency. Therefore, vitamin supplementation may be beneficial in improving health outcomes in women with PCOS and obesity.

This study is the first MR investigation exploring the association between vitamin supplementation and PCOS and its key pathways. The MR design strengthens causal inference by reducing residual confounding and other biases. The use of data obtained from independent large GWAS ensures the reliability of the results. Furthermore, to address the potential influence of pleiotropic SNPs on our data, we implemented various techniques such as weighted median and MR-RAPS to minimize violations of the MR assumptions. Additionally, we used MR-PRESSO to identify and assess the probable presence of pleiotropy among the SNPs. Lastly, the genetic variants used as IVs were located on different chromosomes, minimizing the potential gene-gene interactions in our findings.
Nevertheless, our study has some limitations. First, the vitamin levels analyzed were genetically predicted concentrations, approximating average effects over the life course. The concentration of vitamins is influenced by the diet. Second, the analysis was restricted to participants of European ancestry to minimize bias due to population stratification; this limits the generalizability of the findings to non-European populations. Third, weak instrument bias may be present, given the low variability of vitamin levels explained by the SNPs. Fourth, although our study incorporates data from extensive genetic epidemiology networks, it may not be adequately powered to detect considerably small effects. Fifth, we were unable to obtain data stratified by the PCOS phenotype, warranting further investigation into the effect of vitamin supplements on different PCOS phenotypes. Lastly, we were unable to assess linear associations between vitamin levels and PCOS. Further prospective and functional studies are warranted to elucidate the role of vitamin supplements in PCOS.

CONCLUSION
Our MR analysis suggests that higher levels of vitamins A, D, E, K, and B-12 are causally related to a reduced risk of PCOS or its key pathways. Further prospective population-based studies and in vivo and in vitro trials are required to clarify the precise function of vitamin supplements in the onset of PCOS and its key pathways.

ARTICLE HIGHLIGHTS
Research background
Outcomes from conventional observational investigations are based on the limited sample size, and influenced by confounding factors.

Research motivation
To conduct a two-sample MR analysis to assess the impact of plasma levels of vitamins A, B-12, D, E, and K on PCOS and its key pathways, namely insulin resistance, hyperlipidemia, and obesity.
**Research objectives**
To explore the causal relationship between increased vitamin A, D, E, K, and B-12 values and a reduced risk of PCOS or its primary pathways.

**Research methods**
The inverse variance weighted (IVW) method is considered highly reliable when there is no evidence of directional pleiotropy among the selected IVs. Complementary analyses were conducted using the weighted median and MR-Egger methods as supplements to IVW. Furthermore, the MR-Robust adjusted profile score (MR-RAPs) and MR-PRESSO approach was used to identify outlier SNPs and provide causal estimations after removing probable outliers, assuming that the employed SNPs are valid.

**Research results**
This MR analysis, based on large-scale genetic consortia, provides suggestive evidence supporting a causal effect of higher vitamin E and B-12 Levels on a decreased risk of PCOS. Our findings indicated that genetically predicted levels of vitamin K and B-12 were related to a lower risk of obesity. Additionally, genetically predicted higher levels of vitamin E, D, and A were suggestively associated with a decreased risk of hyperlipidemia, while higher vitamin E levels were suggestively linked to a lower risk of insulin resistance.

**Research conclusions**
Higher levels of vitamins A, D, E, K, and B-12 are causally related to a reduced risk of PCOS or its key pathways.

**Research perspectives**
Further prospective population-based studies and *in vivo* and *in vitro* trials are required to clarify the precise function of vitamin supplements in the onset of PCOS and its key pathways.

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